

## ARTHROPOD SUCCESSION IN RATS EUTHANIZED WITH CARBON DIOXIDE AND SODIUM PENTOBARBITAL

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**Abstract.**—Arthropod succession was observed on whole rat carcasses euthanized by CO<sub>2</sub> anoxia and sodium pentobarbital (SP) overdose. Adult Diptera accounted for 84% (417/494) of all arthropods collected of which 76% (319/417) were Calliphoridae. Calliphorid oviposition on SP euthanized rats was protracted throughout the first 7 d of putrefaction compared to CO<sub>2</sub> euthanized rats. Seventy-one percent (353/494) of adult arthropods were associated with SP euthanized rats and SP rats took twice as long to decompose. Arthropod succession and development on rat carcasses was most likely influenced by manner of death.

**Key words:** forensic entomology.

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Cadavers exposed to air undergo a series of decompositional stages and arthropods characteristic of each stage appear in regular succession (Keh, 1985). Forensic entomologists frequently assist homicide investigations in determining the postmortem interval (PMI) or time interval between death and corpse discovery based on the diversity of arthropods present and their developmental stage relative to prevailing environmental conditions.

Manner of death can affect the rate of decomposition and arthropod succession and should be considered when using insects to determine the PMI (Smith, 1976; Catts, 1992). Insects associated with poisoned cadavers arrive and develop at different rates than those associated with mechanical deaths (Utsumi et al., 1958). Poisons can deter early invasion by ovipositing flies, larval feeding and beetle predation and inhibit organisms that enhance decomposition (Glaister and Rentoul, 1966; Lane, 1975; Nuorteva and Nuorteva, 1982; Mann et al., 1990; LeClerq and Vallant, 1992).

Substance abuse from prescription and non-prescription drugs often cause or contribute to the death of an individual. Drugs can be detected in decomposing tissues and in the maggots feeding on such tissues as reviewed by Goff and Lord (1994). The presence of drugs in decomposing tissues also influences the development and pattern of carrion feeding insects and could alter PMI estimates based on the rate of larval and puparial development. Lord (1990) described a homicide case in which a single *Phaenica serricata* (Meigen) larva recovered from the nasal region of a woman with a history of cocaine abuse had undergone accelerated growth and was nearly twice the size of other *P. serricata* and *Cynomyopsis cadaverina* (R.-D.) larvae that were present. In laboratory studies, sarcophagid fly colonies developed more rapidly on tissues containing cocaine, heroin (as morphine) and metamphetamine (Goff et

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al., 1989, 1991, 1992). Conversely, the developmental rate of *Parasarcophaga ruficornis* (F) feeding on tissues containing the antidepressant amitriptyline was not affected however, a prolonged postfeeding period was observed (Goff et al., 1993).

The studies by Goff et al. (1989, 1991, 1992, 1993) used livers and spleens from rabbits receiving known dosages of cocaine, heroin, metamphetamine and amitriptyline as larval media and sarcophagid flies as primary necrophages. The objective of our study was to compare arthropod succession and carcass decomposition in whole rats euthanized by CO<sub>2</sub> anoxia and sodium pentobarbital (SP) overdose under environmental conditions common to central New York. SP, a barbituric acid derivative, is used as a veterinary anesthetic and euthanasia drug. Carbon dioxide euthanasia was selected for comparison to SP overdose because in both cases death results from anoxia (Muir III and Hubbell 1995).

#### MATERIALS AND METHODS

Five rats were euthanized in a CO<sub>2</sub> chamber and 5 rats were euthanized by intraperitoneal injection of 1.5 cc SP (Nembutal: 64.8 mg SP/cc 10% ethanol in water). Rats weighed 0.25 to 0.40 kg and were culled from a Cornell University nutrition study. Death occurred within seconds for rats receiving SP and within 5 mins for rats euthanized in the CO<sub>2</sub> chamber. One to 4 h after death, rats were placed in 29 × 36 × 54 cm wire cages 7 m apart in open pasture behind Schwardt Laboratory, Ithaca, NY. Rats were euthanized and placed in the field on Sept. 13, 1991 (1 CO<sub>2</sub> rat per cage and 1 SP rat per cage) and October 1 and 18, 1991 (2 CO<sub>2</sub> rats per cage and 2 SP rats per cage). Rainfall and afternoon temperature were recorded for 21, 17, and 15 days following placement of the rats in the pasture on Sept. 13, Oct. 1 and 18, respectively.

Diptera and other flying insects, fly larvae and beetles were collected daily as described by Lord and Burger (1983). Although not collected, other insects seen associated with the rat carcasses were recorded in a field notebook. No collections were made after sunset. Time required for fly larval and pupal development were also recorded. Arthropods were identified to family although some calliphorid flies, beetles and yellowjackets were identified to genus and occasionally species. Arthropod diversity and succession were correlated to the stages of decomposition and method of euthanasia. Data were analyzed by *t* test (Systat, 1992).

#### RESULTS

*Sept. 13.* Within 1 d postmortem blow fly (Calliphoridae) oviposition was extensive for both CO<sub>2</sub> and SP euthanized rats. For brevity, rats will be referred to as CO<sub>2</sub> and SP rats. Eggs were laid within the nostrils, along the genito-anal areas and along the flanks. The SP rat had eggs in its eyelids and yellowjackets (Vespidae: *Vespula maculifrons* [Buysson]) feeding at the scrotum. The left flank of the CO<sub>2</sub> rat was torn by yellowjackets.

Two d postmortem 0.5–0.7 cm maggots had entered the left flank of the CO<sub>2</sub> rat, eaten through the peritoneum and entered the viscera. The eyes were eaten and nose blood had congealed. Maggots measuring 0.3–0.5 cm on the SP rat were feeding around the face and rectum. A 1 cm larva was noted deep within the perineoscrotal cavity but was not removed. Fly larvae were not detected within the abdomen.

Yellowjackets contributed to carrion decomposition by opening the carcass to other insects.

Three d postmortem 1.1–1.4 cm second and third instars were actively feeding on the intestines, gall bladder, abdominal mesentery, face, mouth and lips of the CO<sub>2</sub> rat. Postfeeding third instars were beginning to leave the corpse. The SP rat was distended and first and second instars were on the nape of the neck, ears, eyes and perineum.

Four d postmortem all viscera of the CO<sub>2</sub> rat were eaten. The bones, hair and papery skin remained. Fly larvae had tunneled into the ground and predators such as: histerid (Histeridae) beetles, rove (Staphylinidae) beetles, sphecid (Sphecidae) wasps and assassin (Reduviidae) bugs dominated. The SP rat was bloated and second instars began feeding on the intestines, liver and kidneys. The largest fly larvae, 1.2–1.5 cm, fed within the perineoscrotal cavity.

Six d postmortem the CO<sub>2</sub> rat was dry. Ants (Formicidae), opilionids (Opiliones) and entomobryiid (Entomobryidae) collembolans scavenged. Much of the SP rat's esophagus and gastrointestinal tract were still intact. The next 5 d exhibited little change in the CO<sub>2</sub> rat whereas larval and adult calliphorids still dominated the SP rat.

The mean afternoon temperature from Sept. 13–Sept. 24 was 24°C (15–36°C). Average rainfall was 0.25 cm. Arthropods associated with the rats described above are listed in Table 1.

*Oct. 1.* All four CO<sub>2</sub> and SP rats had extensive oviposition near their perianal areas, mouths, necks and flanks within 1 d of death. Adult calliphorid flies were feeding near the rectum and face.

Within 3 d postmortem the CO<sub>2</sub> rats' eyes were liquefied by feeding fly larvae. First instars fed in the nose, flanks and genitourinary system of SP rats. The calliphorid flies *Lucilia* sp. and *Phaenicia* sp. continued to oviposit on the SP rat.

Within 6 d postmortem the CO<sub>2</sub> rats' faces were eaten and their mandibles were free, skin was blackened, fur fell off easily and larvae, having consumed the viscera, started eating the muscles. Staphylinid and histerid beetles also frequented the carcasses. In contrast, the SP rats were still intact, their flesh was pink and fly larvae were concentrated in the genito-anal area and upper thorax. Larval movement appeared somewhat languid in SP rats compared to similar instars in CO<sub>2</sub> rats.

Nine d postmortem the CO<sub>2</sub> rats' brains were eaten, their skins were tight, their thoraces deflated and postfeeding third instars were tunneling in preparation for pupation. The faces and viscera of SP rats were still intact and all fly instars were present. SP rats were mauled by vertebrates, most likely raccoons, 10 d postmortem. Over the next 6 d we observed additional desiccation, melanization and yellowing of the CO<sub>2</sub> rat carcasses. Seventeen d postmortem the CO<sub>2</sub> rats also were eaten by vertebrates.

The mean afternoon temperature from Oct. 1–Oct. 17 was 18°C (9–27°C). Average rainfall was 0.53 cm. Arthropods associated with these four rat carcasses are listed in Table 1. The number of taxa observed or collected from SP rats was significantly greater than the taxa associated with CO<sub>2</sub> rats ( $P < 0.05$ ). The largest number of taxa were observed or collected from Sept. 13 SP rats 4–6 d postmortem ( $P < 0.05$ ).

*Oct. 18.* The last four rats were never fully decomposed by arthropods. Four d postmortem the 2 SP rats and 1 CO<sub>2</sub> rat were eaten by vertebrates. The four rats

Table 1. Adult arthropod succession to rats euthanized by CO<sub>2</sub> anoxia and sodium pentobarbital (SP) overdose.

Days postmortem	Order <sup>a</sup>	September 13, 1991			October 1, 1991		
		CO <sub>2</sub>	SP	CO <sub>2</sub>	SP	SP	
1	Diptera	Sarcophagidae	Calliphoridae	Calliphoridae	Calliphoridae	Calliphoridae	
		Calliphoridae					
	Hymenoptera	Ephyridae	Vespidae			Sphexidae	
	Coleoptera	Vespidae	Staphylinidae				
2	Diptera	Formicidae					
		Collembola		Opiliones		Opiliones	
	Hymenoptera	Sarcophagidae	Sarcophagidae	Calliphoridae	Calliphoridae	Calliphoridae	
		Calliphoridae	Calliphoridae	Calliphoridae	Calliphoridae	Calliphoridae	
3	Diptera	Vespidae	Vespidae	Calliphoridae	Calliphoridae	Calliphoridae	
		Formicidae					
	Hymenoptera	Formicidae	Staphylinidae				
		Opiliones		Opiliones		Opiliones	
4	Diptera	Collembola				Collembola	
		Sarcophagidae	Sarcophagidae	Calliphoridae	Calliphoridae	Calliphoridae	
	Hymenoptera	Calliphoridae	Calliphoridae	Calliphoridae	Calliphoridae	Calliphoridae	
		Sphaeroceridae	Sepsidae	Sarcophagidae	Sarcophagidae	Sarcophagidae	
5	Diptera	Muscidae	Muscidae	Muscidae	Muscidae	Muscidae	
		Braconidae	Anthomyiidae				
	Hymenoptera	Formicidae	Formicidae	Formicidae	Formicidae	Formicidae	
		Staphylinidae	Staphylinidae				
6	Diptera	Gryllidae	Gryllidae				
		Opiliones	Opiliones				
	Hymenoptera	Sarcophagidae	Sarcophagidae	Calliphoridae	Calliphoridae	Calliphoridae	
		Calliphoridae	Calliphoridae	Calliphoridae	Calliphoridae	Calliphoridae	
7	Diptera	Muscidae	Muscidae				
		Formicidae	Formicidae				
	Hymenoptera	Staphylinidae	Staphylinidae				
		Opiliones	Opiliones				

Table 1. Continued.

Days postmortem	Order <sup>a</sup>	September 13, 1991			October 1, 1991		
		CO <sub>2</sub>	SP	CO <sub>2</sub>	SP	SP	
5	Hymenoptera	Sphecidae	Sphaeroceridae				
			Chloropidae				
			Vespidae				
	Coleoptera	Staphylinidae Histeridae	Formicidae		Formicidae		
			Histeridae		Staphylinidae Histeridae		
			Siphilidae				
	Hemiptera Orthoptera Other	Reduviidae	Staphylinidae				
			Reduviidae				
			Gryllidae				
	Diptera		Acari			Collembola Calliphoridae	
			Sarcophagidae				
			Calliphoridae				
6	Hymenoptera Coleoptera	Formicidae Staphylinidae	Muscidae				
			Phoridae				
			Sciariidae				
	Hemiptera Other Diptera	Collembola Sarcophagidae	Sphaeroceridae				
			Formicidae				
			Staphylinidae		Staphylinidae		
	Hymenoptera Coleoptera Orthoptera	Formicidae	Staphylinidae				
			Chrysomelidae				
			Calliphoridae			Reduviidae Collembola No collecting	
	Hymenoptera Coleoptera Orthoptera	Formicidae	Sepsidae		Opiliones No collecting		
			Lonchopteridae				
			Braconidae				
Hymenoptera Coleoptera Orthoptera	Formicidae	Staphylinidae					
		Gryllidae					
		Gryllidae					



Table 1. Continued.

Days postmortem	Order <sup>a</sup>	September 13, 1991		October 1, 1991	
		CO <sub>2</sub>	SP	CO <sub>2</sub>	SP
7	Other	Opiliones	Acar	Psychodidae	Sciariidae
	Diptera	Muscidae Sphaeroceridae	Muscidae		
	Hymenoptera		Braconidae	Staphylinidae	Staphylinidae
	Coleoptera Orthoptera Other	Collembola	Gryllidae	Collembola	Collembola Opiliones
8	Diptera	Sphaeroceridae	Calliphoridae Muscidae	Calliphoridae Sphaeroceridae	Calliphoridae Sarcophagidae Sphaeroceridae Staphylinidae Braconidae
	Coleoptera				
	Hymenoptera				
	Orthoptera Other	Collembola	Gryllidae	Collembola	
9	Diptera		Muscidae	Sphaeroceridae	Staphylinidae
	Coleoptera			Staphylinidae	Braconidae
	Hymenoptera				
	Orthoptera Diptera	Sarcophagidae			
10	Coleoptera				
	Hymenoptera		Formicidae		
	Orthoptera		Gryllidae		
	Diptera		Sarcophagidae Calliphoridae Sepsidae Sphaeroceridae Staphylinidae Formicidae		
11	Coleoptera				
	Hymenoptera	Formicidae			
	Other	Collembola			
	Diptera	Muscidae	No arthropods		

days 9-17

Table 1. Continued.

Days postmortem	Order <sup>a</sup>	September 13, 1991		October 1, 1991	
		CO <sub>2</sub>	SP	CO <sub>2</sub>	SP
12	Hymenoptera	Formicidae			
	Other	Collembola			
	Diptera	Sphaeroceridae	Sphaeroceridae		
	Other	Collembola Acari	Araneae		
13	Diptera		Sphaeroceridae		
to	Hymenoptera		Formicidae		
39	Coleoptera		Staphylinidae		
	Other	Collembola	Collembola		

<sup>a</sup> Taxa listed in order of abundance.

Table 2. Adult arthropods collected from rats euthanized by CO<sub>2</sub> anoxia and sodium pentobarbital (SP) overdose.

Taxa	Adults collected	Percent on CO <sub>2</sub> rats	Percent on SP rats	Percent of total
Diptera	417	27.3	72.7	84.4
Coleoptera	27	51.9	48.2	5.5
Hymenoptera	20	30.0	70.0	4.1
Hemiptera	6	16.7	83.3	1.2
Others	24	25.0	75.0	4.9
Total	494	28.5	71.5	100

were visited by calliphorid (*Lucilia* sp., *Phaenicia* sp. and *Phormia* sp.), sarcophagid (*Sarcophaga* sp.) and muscid (Muscidae) flies; braconid (Braconidae) wasps, collembolans, ants and opilionids. The remaining CO<sub>2</sub> rat was observed for 15 d.

Twelve d postmortem distention waned in the remaining CO<sub>2</sub> rat and calliphorid larvae were seen feeding around the vaginal and anal area. Three d later, this rat was still intact, although a putrid smell was evident. The mean afternoon temperature for Oct. 18–Nov. 1 was 15°C (7–23°C). Average rainfall was 0.03 cm and 6 of the 15 d were overcast. The few arthropods associated with the Oct. 18 rats are not included in Table 1.

*Fauna.* A total of 494 adult arthropods were collected from the seven rats, 28.5% from the four CO<sub>2</sub> rats and 71.5% from the three SP rats (Table 2). Except for the Coleoptera, the majority of adult arthropods were collected from the SP rats compared to CO<sub>2</sub> rats ( $P < 0.05$ ). Diptera accounted for 84% (417/494) of the adult arthropods collected ( $P < 0.05$ ) and 76% (319/417) were Calliphoridae. The predominant calliphorids were *Phaenicia* sp., *Opsodexia* sp., *Lucilia illustris* (Meigen), *Phormia regina* (Meigen) and *Cochliomyia macellaria* (F.). Muscidae accounted for 5.7% (24/417) and Sarcophagidae accounted for 3.4% (14/417) of the Diptera. The remaining families: Sphaeroceridae, Chloropidae, Sepsidae, Lonchopteridae, Phoridae, Ephydriidae, Sciaridae and Piophilidae accounted for 14.4% (60/417) of the Diptera.

Seventy-four percent (20/27) of the adult Coleoptera were staphylinids. The genera *Philonthus* sp., *Aleochara* sp., *Omalium* sp. and the predatory species *Creophilus maxillosus* (L.) were predominant. Other beetles associated with the rat carcasses were Histeridae, incidental leaf beetles (Chrysomelidae) and the carrion beetles *Silpha* sp. and *Nicrophorus* sp., the latter carrying numerous phoretic *Poecilochirus* sp. mites.

#### DISCUSSION

Both groups of rats attracted similar arthropods. Diptera accounted for 84% (417/494) of collected adults with Coleoptera, Hymenoptera, Hemiptera and others representing between 1–5.5% each. Other arthropods included acarines (Acari), opilionids (Opiliones), gryllids (Gryllidae), collembolans and spiders (Araneae). The number of taxa were fairly constant for CO<sub>2</sub> rats but diverse at the beginning and poor at the end for SP rats. Seventy-one percent (353/494) of adult arthropods were associated with the SP rats.



CO<sub>2</sub> rats decomposed as expected for small rodents (Lane, 1975). Adult insect activity increased from day 2–3, decreased from 4–5 and stabilized after day 5. The SP rats exhibited heightened adult activity from d 2–5 and did not exhibit significant reduction in insect activity until day 7. Increased fly activity on d 2 for both CO<sub>2</sub> and SP rats followed the initial calliphorid oviposition and was comprised of sarcophagids, sphaerocerids, muscids, chloropids, piophilids, sepsids and ephydriids. The CO<sub>2</sub> rats decomposed in half the time as SP rats and were unavailable for additional waves of opportunistic feeders and facultative necrophages. By comparison, when only the bones, hair and skin of the CO<sub>2</sub> rats remained, the SP rats were bloated with feeding second instars. Even 9 d postmortem first instars were detected in SP rats. Calliphorid oviposition on SP rats appeared protracted throughout the 7 d of putrefaction.

Although we did not test for the presence of SP in decomposing rat tissues or in the carrion feeding insects, our results indicate that SP affects fly oviposition and feeding patterns. The presence of SP in the tissues may have inhibited fly larval feeding and development particularly in the abdominal region where the highest concentrations of SP residues would be expected following an intraperitoneal injection. We found Diptera larval feeding concentrated in the genito-anal area, upper thorax and face of the SP rats while most of the gastrointestinal tract and abdominal mesentery was uneaten. Delayed oviposition and larval feeding and development were observed on mercury contaminated fish tissues (Nuorteva and Nuorteva, 1982) and on malathion contaminated human tissues (Gunatilake and Goff, 1989).

Smith (1976) stressed the need for faunal succession studies on whole animal carcasses because similar studies on intact human corpses under field conditions were not ethically or morally possible. Methods used to euthanize animals in experimental studies affect faunal succession as does the kind and size of the animal used (Denno and Cothran, 1975; Hewadikaram and Goff, 1991). For instance, carbon monoxide gas changes the blood hemoglobin which alters body tissues and affects the rate of decomposition and carcasses with artificial wounds would desiccate more rapidly and affect results (Smith, 1976). We compared rats of similar weight euthanized by CO<sub>2</sub> anoxia and SP overdose which also kills by anoxia. Death by cervical fracture occurs through the elimination of the brain blood supply and central nervous system input and is not equivalent to SP euthanasia. Furthermore, cervical fracture is not suitable for animals >100 gm (Muir III and Hubbell, 1995). Although a rise in blood pH would be expected from both CO<sub>2</sub> and SP euthanasia, the latter due to glycolysis after death, CO<sub>2</sub> leaves no residues while SP, which is highly lipid soluble, is concentrated in the fatty tissues. In fact, Beyer et al. (1980) detected phenobarbital, a related barbiturate, in *C. macellaria* larvae feeding on the decomposed remains of a fatal overdose case.

Care must be taken in estimating PMIs from insects feeding on corpses containing poison, drug and toxin residues. Jirón and Cartín (1981) observed insect succession on a dog euthanized with SP during the dry season in Costa Rica. The ecological complexity and fauna associated with the dog's decomposition were reportedly due to the premontane humid climate. Unfortunately, the authors did not consider manner of death in their study of insect succession. Utsumi et al. (1958) identified insects attracted to rats euthanized nine different ways. Insect succession to poisoned rats was fairly constant however, rats euthanized mechanically exhibited plentiful insect

activity at first that later declined. We observed prolonged adult activity and larger numbers of adults on SP rats most likely because SP rats took twice as long to decompose as CO<sub>2</sub> rats.

Although manner of death influences insect succession and development of sarcophagous insects (Smith, 1976). Rats euthanized and placed outside on Sept. 13 decomposed twice as fast as rats euthanized and placed outside on Oct. 1. The mean temperature from Sept. 13–24 was 24°C whereas the mean temperature from Oct. 1–17 was 18°C. Higher temperatures in September accelerated fly larval development and enhanced bacterial putrefaction. Adult Diptera were more numerous in September than October. Two d postmortem a 1 cm larva was found within the perineo-scrotal cavity of the Sept. 13 SP carcass. This anomaly may be explained by localized larval mass heat generation or by early hatching. The predominance of calliphorid, sarcophagid, muscid, piophilid, sepsid and phorid larvae in the September rat carcasses increased the rate of decomposition. Although interesting, many of these synanthropic flies are not encountered in typical forensic investigations (Catts and Haskell, 1990). Facultative necrophages, such as Collembola, ants, opilionids, crickets and yellowjackets also were more abundant in September than in October.

Conspicuously missing are the Coleoptera families Trogidae, Dermestidae, Cleridae, and Nitidulidae that are frequently collected from advanced decay and dry stages of carrion (Payne and King, 1970; Smith, 1986). Nocturnal beetles may have been missed because we collected during the day. Other beetles may have been inhibited by the truncated photoperiod of September and October, sporadic rain, the small size of the rat carcasses or an unsuitable biochemical microclimate, all factors to consider in forensic entomology studies.

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